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TITLE: Eliciting Autoimmunity to Ovarian Tumors in Mice by Genetic Disruption of T Cell Tolerance Mechanisms

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13. ABSTRACT (Maximum 200 Words)
We are developing a mouse model for ovarian cancer that will allow monitoring of the in vivo activities of tumor-specific T cell clones as they encounter ovarian tumors in vivo. We propose to "tag" the neu oncogene with two identified T cell epitopes so as to confer recognition by available T cell receptor (TCR) transgenic T cells. When expressed in the murine ovarian tumor cell line ID8, epitope-tagged neu (designated $neu^{OT1/OT2}$) should induce formation of aggressive ovarian adenocarcinomas that express the epitope tags and hence are recognizable by adoptively transferred TCR transgenic T cells. We successfully made the $neu^{OT1/OT2}$ expression construct, but found it to be overly immunogenic in vivo such that tumors were spontaneously rejected. Therefore, we derived a third generation ID8 tumor cell line that has a shorter tumor latency and decreased expression of MHC Class I, which should make it less immunogenic. Meanwhile, we have commenced adoptive T cell transfer experiments using a convenient, transplantable lymphoma model. With this model, we are investigating the phenomenon of antigen spreading that results after adoptive transfer of tumor-specific T cells. Finally, Cbl-b -/- mice have been obtained and are currently being backcrossed onto the B6 background for Aim 3.

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DAMD17-01-1-0733 Annual Progress Report 2003

PI: Brad H. Nelson, Ph.D.

Title of Project: Eliciting Autoimmunity to Ovarian Tumors in Mice by Genetic Disruption of T Cell Tolerance Mechanisms

Introduction:

Research in the fields of basic immunology and autoimmunity has identified several distinct mechanisms through which immune tolerance is established and maintained in the normal host, and additional mechanisms will likely be identified in future. We hypothesize that ovarian tumors are recognized in an antigen-specific manner by T cells but induce immunologic tolerance through one or more of these homeostatic mechanisms, which have evolved to protect the host from autoimmune attack. We further hypothesize that tolerance to ovarian tumors can be overcome by disrupting critical components of tolerogenic pathways through genetic manipulation of T cells. To test this hypothesis, we proposed to develop a murine model for ovarian cancer that will allow, for the first time, precise monitoring of the functional responses of naïve, tumor-specific CD4+ and CD8+ T cell clones to ovarian tumors. Multiple properties of tumor-reactive T cells will be assessed *in vivo*, including their localization, activation, anergic status, proliferation and apoptosis. Differential responses and anti-tumor activities of the CD4+ and CD8+ T cell subsets will be investigated. Finally, the model will be used to evaluate the functional responses of tumor-specific CD4+ and CD8+ T cells that are genetically pre-disposed to autoimmune activity. The first tolerogenic pathway tested will be that involving the Cbl-b gene, as T cells lacking Cbl-b have a greatly reduced requirement for CD28 co-stimulation and demonstrate hyperactivity in vivo with profound autoimmune sequelae. The specific aims of this proposal are:

Aim 1. To generate an ovarian tumor cell line that is recognized by antigen-specific CD4+ and CD8+ T cell clones from TCR transgenic mice.
Aim 2. To define the mechanisms by which ID8 ovarian tumors evade rejection by tumor-specific CD4+ and CD8+ T cells.
Aim 3. To determine whether tumor-specific CD4+ and CD8+ T cells lacking the Cbl-b gene show enhanced functional responses to ovarian tumors.

Body:

Aim 1: To generate an ovarian tumor cell line that is recognized by antigen-specific CD4+ and CD8+ T cell clones from TCR transgenic mice.

As described in the last progress report, it proved necessary to generate a more aggressive ovarian tumor cell line for our experiments, as we encountered problems with spontaneous rejection of the original ID8 cell line. A more aggressive subclone was successfully derived via two rounds of serial passage in vivo. The new subclone induces tumors in just 30-40 days, as opposed to the 120-day latency of the original ID8 cell line. We have successfully transfected the new subclone with the $neu^{OT1/OT2}$ construct and achieved stable expression. Next, the transfected cells will be injected intraperitoneally into syngeneic female mice to confirm that the cells have retained their tumorigenic properties. This step has been delayed due to the PI's recent move to the B.C. Cancer Agency. The PI's new lab will not be operational until October 2003, and furthermore, it has been necessary to apply for ACUC approval at the new institution. While no major problems are expected, these move-related issues have nevertheless caused a significant delay in the project's timeline. Please note that these issues have been discussed with and approved by Dr. Bora. A new timeline was submitted to Dr. Bora, and this has been incorporated into the current progress report.

Aim 2: To define the mechanisms by which ID8 ovarian tumors evade rejection by tumor-specific CD4+ and CD8+ T cells.

While we have been developing the optimal ID8 subclone to use for these studies (Aim 1), we have forged ahead with adoptive T cell transfer experiments, similar to what was described in Aim 2 of the original

proposal. Our goals are two-fold: (1) to hone our skills at adoptive T cell transfers and flow cytometry, which are technically demanding, and (2) to further refine the experimental questions that will eventually be addressed in the ovarian tumor model. To this end, we have used a well-characterized lymphoma model involving the EL-4 cell line transfected to express the model antigen chicken ovalbumin (OVA). The OVA antigen is recognized by the OT-I (CD8+) and OT-II (CD4+) T cells described in the original proposal.

To determine if OT-I and OT-II T cells are capable of eradicating OVA+ lymphoma cells, we adoptively transferred a 50:50 mixture of OT-I and OT-II T cells into mice and simultaneously injected (s.q.) the OVA-positive lymphoma cell line EG7. Control mice received OT-I and OT-II T cells with a s.q. injection of OVA-negative tumor cells. Whereas all control mice (6/6) developed palpable tumors after 14 days, 11/12 of the experimental mice fully rejected the OVA-positive tumor cells, with no evidence of tumor formation even 22 days later (Figure 1). Additional experiments in which OT-I or OT-II T cells were infused alone indicated that both the OT-I and OT-II T cells were capable of rejecting OVA-positive tumor cells. Thus, both the CD4+ and CD8+ T cell subsets can reject lymphoma cells upon adoptive transfer.

Interestingly, a significant fraction (3/7) of mice that rejected the OVA-positive lymphoma cells after adoptive transfer of OT-I and OT-II T cells were found to later reject OVA-negative lymphoma cells (Figure 2). This implies that an immune response directed against one antigen (OVA) was able to induce systemic immunity to other antigens expressed by the lymphoma cells. This phenomenon is known as "antigen spreading" and has been documented in various autoimmune diseases. It is considered by many to be a desirable and perhaps necessary feature of effective anti-tumor immunity, and hence we intend to further investigate the conditions that best promote this process. To this end, we have recently found by infusing OT-I or OT-II T cells separately that CD4+ cells (OT-II) are necessary and sufficient to induce antigen spreading. We are also documenting the process of antigen spreading by western blot using serum from the above mice. Blood was drawn at baseline (day 0) and two weeks after EL4 inoculation (day 36). Mouse sera immunoblotted against EL4 lysate demonstrated several novel immunoreactivities that arose as a result of adoptive T cell therapy (Figure 3). These bands likely reflect antigens within the EL4 lymphoma that become recognized as a result of antigen spreading. In future, we will use serological cloning methods to identify these antigens.

The above studies using the lymphoma model have raised many interesting questions about anti-tumor immunity. Ultimately, we intend to pursue these questions also in the ovarian tumor model, once the new lab becomes operational.

Aim 3: To determine whether tumor-specific CD4+ and CD8+ T cells lacking the Cbl-b gene show enhanced functional responses to ovarian tumors.

As described in last year's progress report, the Cbl-b -/- mice we received from Dr. Josef Penninger's lab were not on a pure B6 background. Therefore, we have had to backcross the mice onto the B6 background. We are currently in our sixth generation of backcrossing, so should be able to perform these experiments in the near future, once the new lab is operational.

Key Research Accomplishments:

The following items have been completed or are underway:

Task 1. To generate an ovarian tumor cell line that is recognized by antigen-specific CD4+ and CD8+ T cell clones from TCR transgenic mice (July 2003-Dec 2003).

a. Evaluate signaling and transforming properties of epitope-tagged and untagged version of *neu* in cell lines; if problems noted, modify epitopes as needed. ***completed***

b. Generate ID8 cell subclones that stably express $neu^{OT1/OT2}$; perform in vitro assays to evaluate recognition of OT1 and OT2 epitopes by CD4+ and CD8+ T cells from TCR-transgenic mice. ***completed***

c. Inject ID8/*neu*$^{OT1/OT2}$ cells intraperitoneally into mice to confirm retention of tumorigenic properties and determine optimal level of expression of *neu*$^{OT1/OT2}$ (July 2003-Dec2003). ***in progress***

Task 2. To define the mechanisms by which ID8 ovarian tumors evade rejection by tumor-specific CD4+ and CD8+ T cells (Jan 2004-July 2005).

a. Generate sufficient numbers of mice bearing tumors expressing *neu*$^{OT1/OT2}$. ***in progress***

b. Perform immunological studies of adoptively transferred OT1- and OT2-specific T cells and control T cells in mice bearing ovarian tumors expressing *neu*$^{OT1/OT2}$, as per Aim 2. ***in progress using a lymphoma model***

Task 3. To determine whether tumor-specific CD4+ and CD8+ T cells lacking the Cbl-b gene show enhanced functional responses to ovarian tumors (Months 21-36).

a. Breed OT1, OT2, TEa and P14 TCR transgenes onto the Cbl-b background (Months 21-36). ***backcrossing is underway***

b. Generate sufficient numbers of mice bearing tumors expressing *neu*$^{OT1/OT2}$ (Months 21-36). ***not yet applicable***

c. Perform immunological studies of adoptively transferred Cbl-b-deficient OT1- and OT2-specific T cells and control T cells in mice bearing ovarian tumors expressing *neu*$^{OT1/OT2}$ (Months 24-36). ***not yet applicable***

Reportable Outcomes:

Posters:

Identifying the signaling pathways that drive T-cell proliferation in response to tumors. Ryan M. Teague, Richard M. Tempero, and Brad H. Nelson. Era of Hope Department of Defense Breast Cancer Research Program Meeting, Orlando, FL, September 2002.

Primary in vivo expansion of naïve CD8$^+$ T cells in the absence of IL-2 receptor and STAT5 signaling Ryan M. Teague, Richard M. Tempero, and Brad H. Nelson. Annual Meeting of the American Association of Immunologists, Denver CO, 2003.

Invited presentations:

Identifying the signaling pathways that drive T-cell proliferation in response to tumors. Brad H. Nelson. 10th Annual SPORE Investigators Workshop, Chantilly VA, July 2002.

The immune response to cancer. Brad H. Nelson. Annual Meeting of the British Columbia Cancer Agency, Vancouver, BC, Canada, November 2002.

The immune response to cancer. Brad H. Nelson. Fox Chase Cancer Center, Philadelphia PA, November 2002.

Career advancement:

The PI, Brad Nelson, has been appointed Director of the Research Laboratories for the British Columbia Cancer Agency's Vancouver Island Centre (Victoria, BC). His work in this animal tumor model was integral to his success in this competition. He moved there on July 1, 2003. (The DOD has already been informed of this move.)

Conclusions:

The mouse model we are developing should lead to an improved understanding of the immune response to ovarian cancer and may facilitate the development of novel immune-based therapies or immunopreventive strategies for this disease. Toward this goal, we have now created a dually epitope-tagged version of *neu* that is recognized by the appropriate CD4+ and CD8+ T cells. We have also generated a more aggressive ovarian tumor cell line that should overcome the obstacle of spontaneous rejection, which we encountered last year. Adoptive T cell studies have been started using a convenient lymphoma model, and we have preliminary results indicating that adoptive T cell therapy can trigger antigen spreading, an important feature of the anti-tumor response. Finally, we have obtained and are currently backcrossing the Cbl-b -/- mice required for Aim 3. No other changes to the research plan are expected.

References:

None.

Appendices:

See accompanying Figures 1-3.

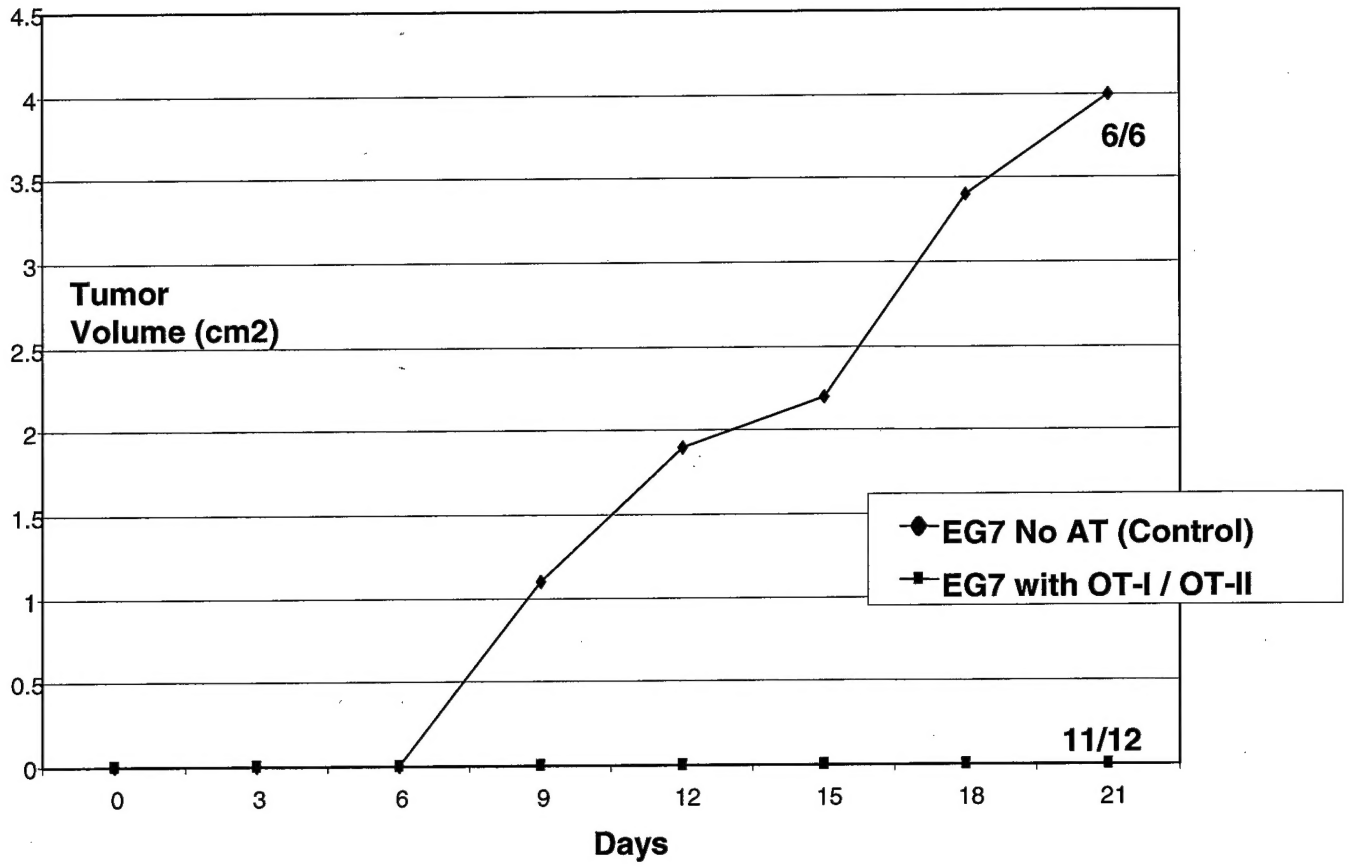

Figure 1. Eradication of OVA-positive lymphoma cells (EG7) by adoptive transfer (AT) of OT-I plus OT-II T cells. Experimental mice (n=12) were infused with a 50:50 mixture of OT-I and OT-II T cells and then innoculated with the OVA-positive tumor cell line EG7. Control mice (n=6) received the EG7 tumor without adoptive transfer of T cells. Tumor volume was monitored for 21 days. The majority (11/12) of mice receiving T cells rejected the EG7 tumor.

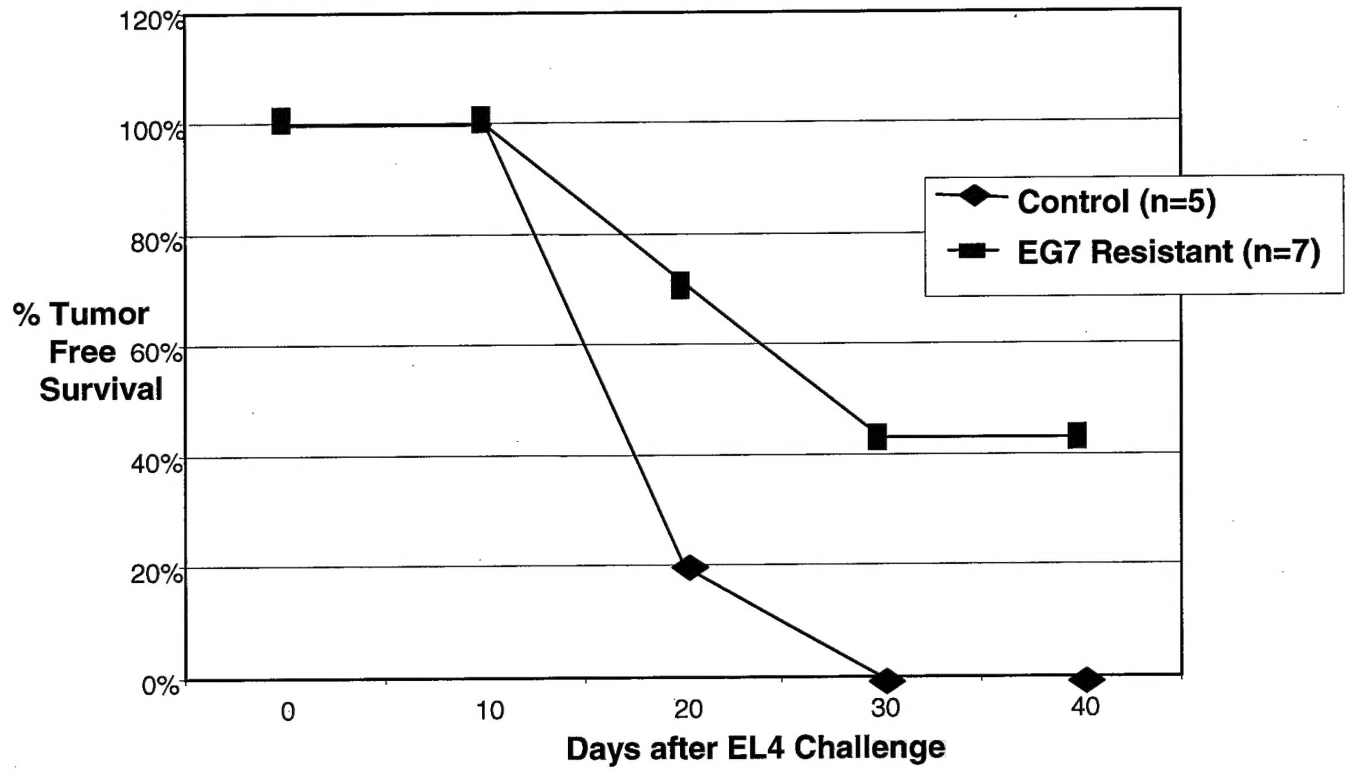

Figure 2. Eradication of OVA-negative EL4 lymphoma cells after prior rejection of an OVA-positive lymphoma by adoptive transfer (AT) of OT-I and OT-II T cells. Twenty two (22) days after eradication of the EG7 tumor, 7 mice were re-challenged with an EL4 tumor in a different location and subsequent tumor growth was monitored. 43% (3/7) mice demonstrated no palpable EL4 tumor up to 40 days post-challenge. By contrast, control mice (n=5) that did not have prior exposure to the EG7 tumor succumbed to the EL4 tumor cell challenge.

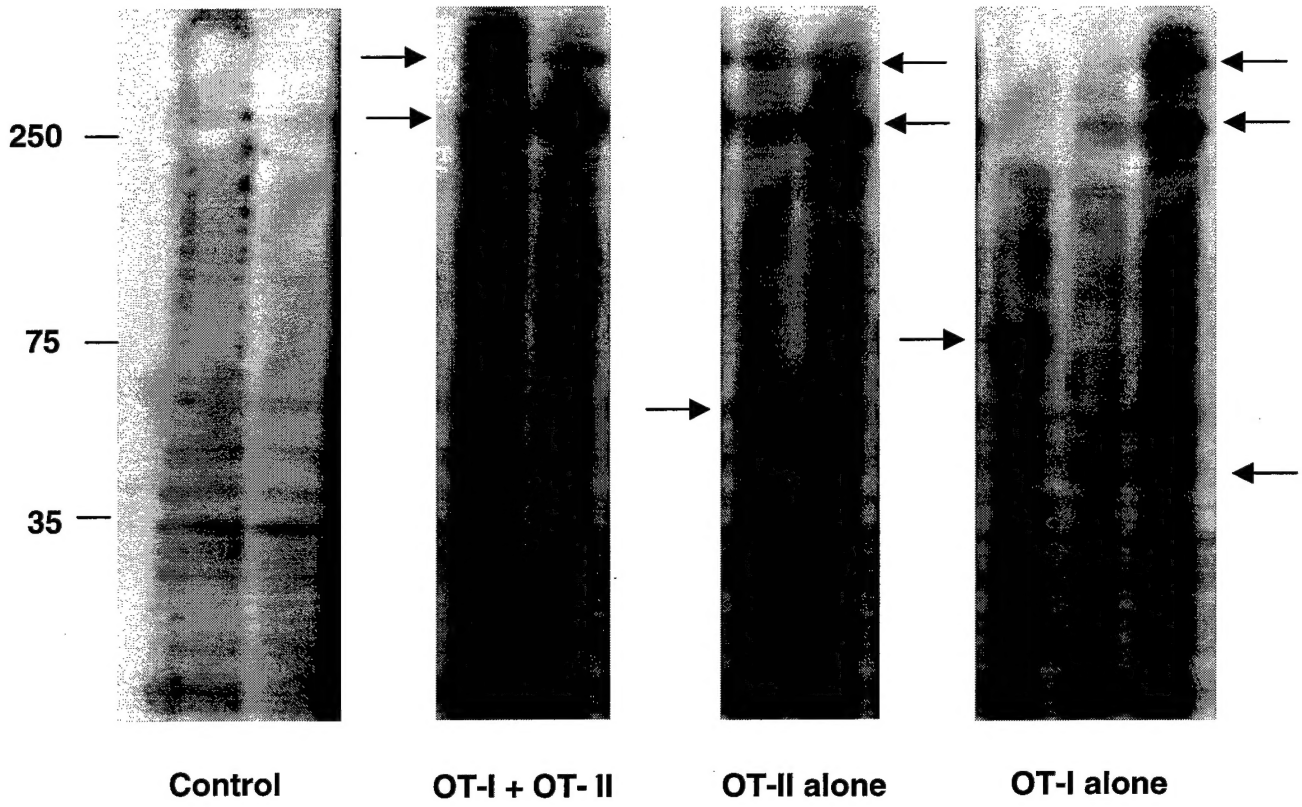

Figure 3. Western blot analysis showing evidence of antigen spreading as a result of adoptive T cell therapy of an EG7lymphoma. Mice were treated as per Figure 2. Thirty six days after challenge with EL4 tumor, serum was harvested from control and experimental mice and used for immunblotting of an EL4 tumor cell lysate. In comparison to control mice, mice that had undergone adoptive T cell therapy with OT-I plus OT-II T cells, OT-I T cells alone, or OT-II T cells alone showed novel antibody responses to various antigens present in EL4 cells (arrows). These antibody responses likely arose by antigen spreading.